Gastrointestial absorption of pimozide is enhanced by inhibition of P-glycoprotein.

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Abstract

Following the death due to cardiac arrest of a patient taking pimozide, sertraline and aripiprazole antipsychotic/antidepressant combination therapy, a role of drug-drug interaction was suggested. Here, we investigated P-glycoprotein (P-gp)-mediated interaction among the three drugs using in vitro methods. Sertraline or aripiprazole significantly increased the permeability of pimozide in Caco-2 cell monolayers. ATPase assay indicated that pimozide is a P-gp substrate, and might act as a P-gp inhibitor at higher concentrations. The values of the kinetic parameters of carrier-mediated efflux, calculated from the concentration dependence of pimozide efflux from LLC-GA5-COL150 cells expressing human P-gp, were as follows: maximum transport rate (J_{max}) = 84.9 ± 8.9 pmol/min/mg protein, half-saturation concentration (K_t) = 10.6 ± 4.7 μM, first-order rate constant (k_d) = 0.67 ± 0.14 pmol/min/mg protein. Further, the efflux ratio of pimozide in LLC-GA5-COL150 cells was significantly decreased in the presence of sertraline or aripiprazole. These results indicate that pimozide is a substrate of P-gp, and its efflux is inhibited by sertraline and aripiprazole. Thus, P-gp inhibition by sertraline and/or aripiprazole may alter the gastrointestinal permeability of co-administered pimozide, resulting in an increased blood concentration of pimozide, which may increase the likelihood of pimozide's known life-threatening side effect of QT prolongation.

Introduction

Pimozide is an antipsychotic used to treat schizophrenic and pediatric autistic disorders. However, one of its major side effects is QT prolongation [1], because it is a strong antagonist of the alpha subunit of a potassium ion channel (hERG) [2] and this action causes a significant prolongation of QT intervals [3]. Several antidepressants are restricted for use in combination with pimozide, because of the increased risk of this side effect [4]. For example, the combination of pimozide and sertraline is contraindicated because it leads to an increased blood concentration of pimozide, thus increasing the risk of QT prolongation. Nevertheless, a case has been reported in which a male child administered pimozide together with sertraline and aripiprazole died due to cardiac arrest [5]. Pimozide is a substrate of the metabolic enzymes CYP3A4, 2D6, and 1A2, and aripiprazole is a substrate of 3A4 and 2D6 [6], while sertraline is a substrate of 2C19, 2C9, 2B6, and 3A4, and a mild to moderate inhibitor of CYP2D6 [7]. Therefore, it had been suspected that interaction among those drugs would be due to inhibition of CYP-mediated pimozide metabolism by sertraline and/or aripiprazole. On the other hand, Alderman reported that the C_{max} and AUC of pimozide were increased by 35% and 37%, respectively, with no significant difference in blood half-life, when the drug was used in combination with sertraline [8]. In pharmacokinetics, blood half-life is inversely proportional to clearance; in other words, if hepatic CYP3A4 and/or CYP2D6 metabolism were inhibited by sertraline, the blood half-life of pimozide should be

prolonged. This suggests that a drug interaction mechanism(s) other than inhibition of CYP3A4 and/or CYP2D6 is involved. We speculated that the drug transporter P-glycoprotein (P-gp) might be an interaction site.

P-gp is a member of the ATP-binding cassette superfamily, and is mainly localized at the intestine, blood-brain barrier, adrenal gland, enterocytes, hepatocytes, placenta and renal proximal tubules in humans [9]. P-gp is responsible for the efflux of many xenobiotics and plays major roles in drug absorption, distribution and excretion. In the intestine, P-gp mediates the efflux of its substrates, restricting the absorption of many xenobiotics, including drugs [10–13]. Recent studies show that sertraline, aripiprazole, and several of their metabolites have a P-gp-inhibitory effect [14–16], whereas pimozide is not known to be a P-gp substrate. The bioavailability of pimozide is limited (about 50%) [17]. Therefore, if pimozide is a P-gp substrate, there is a possibility that its absorption could be increased when it is used in combination with P-gp inhibitors. In order to test this idea, we examined whether pimozide is a P-gp substrate, and whether its gastrointestinal permeability might be influenced by sertraline and/or aripiprazole by means of a series of in vitro studies.

Materials and Methods

Chemicals

Pimozide was purchased from Santa Cruz Biotechnology (Dallas, TX). Sertraline hydrochloide, aripiprazole and verapamil hydrochloride were purchased from Wako Pure Chemical Industries (Osaka, Japan). All other reagents were commercial products of reagent grade.

Cell culture

Caco-2 cells, a human colon epithelial cancer cell line frequently used as an in vitro intestinal model, were purchased from the American Type Culture Collection (Rockville, MD, USA). They were cultured, passaged and grown as described previously [19] in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 0.1 mg/ml streptomycin, and 1.0 % nonessential amino acids, at 37°C in an atmosphere of 5% CO_2 in air. The cells were seeded on Transwell filter membrane inserts (Costar, Bedford, MA, USA) at a density of 6×10^4 cells/cm^2. The culture medium was replaced with fresh medium every second or third day and Caco-2 cell monolayers grown for 21 days were used for the transport studies. Transepithelial electrical resistance (TEER) was measured using a Millicell-ERS resistance system (Millipore, Bedford, MA, USA). Cell monolayers used for transport studies had TEER values of 800 to 1000 $\Omega \cdot cm^2$.

LLC-GA5-COL150 cells, a derivative of kidney epithelial cell line LLC-PK1 expressing human P-gp on the apical membrane, were obtained from Riken Gene Bank (Tsukuba, Japan). They were cultured, passaged and grown as described previously [20] in Medium 199 supplemented with

109 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin and 150 ng/mL colchicine, at
110 37°C in an atmosphere of 5% CO_2 in air. The cells were seeded on Transwell filter membrane inserts
111 (Costar, Bedford, MA, USA) at a density of 2.5×10^5 cells/cm^2 for transport studies [20], and seeded
112 on 24-well cell culture plates (Corning, NY, USA) at a density of 1.5×10^5 cells/cm^2 for efflux assays
113 [21]. The culture medium was replaced with fresh medium every second or third day. Cells were
114 grown for 7 days and used for experiments. One day prior to experiments, the medium was changed
115 to Medium 199 without colchicine. Cell monolayers with TEER values of 300 to 600 Ω·cm^2 were used
116 for transport studies.
117

118 Transport experiments

119 In the transport studies with Caco-2 cell monolayers, the transport medium on the apical
120 side consisted of Hanks' balanced salt solution (HBSS) with 10 mM 2-morpholinoethanesulfonic
121 acid (MES) (pH 6.5) and that on the basal side consisted of HBSS with 10 mM 2-[4-(2-
122 hydroxyethyl)-1- piperazinyl]ethanesulfonic acid (HEPES) (pH 7.4). Test chemicals were dissolved
123 in dimethyl sulfoxide (DMSO) and diluted with transport medium (the final DMSO concentration was
124 1%). Caco-2 cell monolayers were preincubated with the transport medium for 20 min at 37ºC.
125 Transport experiments were initiated by adding the transport medium containing pimozide (10 μM) to
126 the donor side, while the receiver side was filled with transport medium. The concentration of

pimozide was chosen with reference to the clinical dose of 1 mg dissolved in 250 mL of water [22], which corresponds to a concentration of 9 μM. Inhibitor was added to both sides during inhibition studies. Sertraline (500 μM), aripiprazole (15 μM) and verapamil were used as inhibitors (verapamil as a positive control). The tested concentrations of sertraline and aripiprazole were chosen in the same manner as described above, based on the calculation that the clinical doses of sertraline (25 mg) and aripiprazole (3 mg) in 250 mL of water would correspond to concentrations of 292 μM and 27 μM, respectively. Samples were collected at 20, 40, 60, 90 and 120 min, and replaced with equal amounts of transport medium. In the transport studies with LLC-GA5-COL150 cell monolayers, the transport medium on both sides consisted of HBSS with 10 mM HEPES (pH 7.4). Inhibitors were added to the donor side and transport experiments were initiated in the same manner. Samples were collected at 15, 30, 45 and 60 min, and replaced with equal amounts of transport medium. The time course of drug transport in the apical (A) to basal (B) direction (A to B) and that in the opposite direction (B to A) were observed at 37°C. The permeability (P_{app}) across cell monolayers was evaluated by dividing the slope of the experimental time course in the A-to-B or B-to-A direction by the concentration on the donor side and is represented as $P_{app\ AtoB}$ or $P_{app\ BtoA}$, respectively. The efflux ratio (ER) was calculated by dividing $P_{app\ BtoA}$ by $P_{app\ AtoB}$.

ATPase assay

145 The SB-MDR1 PREDEASY™ ATPase Kit, including P-gp-expressing membrane vesicles,
146 was purchased from SOLVO Biotechnology (distributed by KAC Co., Ltd., Kyoto, Japan). This assay
147 kit is designed to measure the interaction of test drugs with P-gp. ATPase assay was conducted
148 according to the manufacturer's instructions and a previous report [18]. Inorganic phosphate liberated
149 by ATP hydrolysis was detected by colorimetric reaction. The optical density (OD) was measured at
150 590 nm with a microplate reader, Sunrise™ Rainbow (TECAN, Kanagawa, Japan).
151

152 Efflux study

153 To investigate the involvement of P-gp in pimozide efflux transport, efflux studies with
154 LLC-GA5-COL150 cells were performed. Cells seeded on 24-well cell culture plates were washed
155 twice with ice-cold Dulbecco's phosphate-buffered saline (-) (D-PBS(-)), and pretreated with 300 μL
156 of ice-cold Opti-MEM containing 0.01 to 100 μM pimozide for 30 min on ice (4℃). The Opti-MEM
157 was removed, and the cells were washed twice with ice-cold D-PBS(-). Experiments were initiated by
158 adding pimozide-free Opti-MEM to each well and incubating the plates at 37℃. After 10 min,
159 transport was stopped by washing each well three times with ice-cold D-PBS(-), and the cells were
160 lysed by adding 200 μL of 0.1 N NaOH solution. Cell lysates were used for protein determination and
161 measurement of pimozide concentration. Protein was determined colorimetrically using DC™ Protein
162 Assay (BIO-RAD, Hercules, CA), based on absorbance measurement at 700 nm with a microplate

reader, Sunrise Rainbow RC (Tecan, Kanagawa, Japan). An aliquot of cell lysate was mixed with 300 μL of ethyl acetate for 10 min in the cold, then 100 μL of the organic phase was moved to a new tube and evaporated. The residue was dissolved in 300 μL of mobile phase. All samples were applied to MultiScreen® Solvent Filter Plates 0.45 μm Low-Binding Hydrophilic PTFE (Merck, Ireland) and centrifuged at 3,500 rpm for 20 min at 4℃. Filtered samples were collected in a 96-well microplate (Asone, Japan), and pimozide concentration was determined by triple quadrupole liquid chromatography mass spectrometry (LC-MS/MS) as described below. Efflux rate was calculated according to the following equation.

Efflux rate = $(X_0 - X_1)$ / incubation time

Where X_0 is pimozide concentration per protein amount before incubation and X_1 is that after incubation. Therefore, $X_0 - X_1$ is the net transport of pimozide from LLC-GA5-COL150 cells.

Kinetic analysis

To estimate the kinetic parameters of carrier-mediated transport in the efflux assays, the transport rate (J) was fitted to the following equation (1), containing saturable and nonsaturable-linear terms, by using the nonlinear least-square regression analysis program, MULTI, as previously reported [23].

$$J = J_{max} \times S/(K_t + S) + k_d \times S \qquad (1)$$

Where J_{max} is the maximum transport rate for the carrier-mediated transport, S is the substrate concentration, K_t is the half-saturation concentration, and k_d is the first-order rate constant.

Measurement of pimozide

Concentrations of pimozide were determined by LC-MS/MS analysis. Pimozide samples (10 μL) were injected into an HPLC system (LC-20A system, Shimadzu, Kyoto, Japan) equipped with a CAPCELL PAK C18 MGⅢ / 3 μm column (ϕ 2.0 × 50 mm, Shiseido Co. Ltd., Tokyo, Japan). The mobile phase consisted of a mixture of acetonitrile containing 0.1% formic acid (organic solvent phase) and distilled water containing 0.1% formic acid (water phase) (50 : 50). The flow rate was 0.1 mL/min, at 40℃. Analytes were detected using a quadrupole mass spectrometer (LCMS-2010EV, Shimadzu) fitted with an electrospray ionization source. Analytes were detected in the positive mode, and the protonated molecular ion of pimozide was monitored at m/z = 109.05.

Statistical analysis

Kinetic parameters are presented as mean ± standard deviation (S.D.). Other data are presented as mean ± standard error of the mean (S.E.M.). Statistical analysis of kinetic parameters was performed by means of Dunnet's *t*-test. A difference between means was considered to be significant when the *P* value was less than 0.05.

Results

Transport study across Caco-2 cell monolayers

The P_{app} of pimozide in the A-to-B direction in the presence of sertraline was 5.9-fold higher than that in the absence of sertraline. The P_{app} in the B-to-A direction was decreased by sertraline, aripiprazole and verapamil, and sertraline had the greatest effect (Fig 1A). Overall, the addition of sertraline, aripiprazole and verapamil decreased the efflux ratio to 2.9 %, 60.6 % and 43.9 % respectively, compared with pimozide alone (Fig 1B).

Fig 1. Effects of sertraline, aripiprazole and verapamil on pimozide transport across Caco-2 cell monolayers.

The permeability of pimozide at a concentration of 10 μM was measured in Caco-2 cell monolayers in the absence or presence of sertraline, aripiprazole or verapamil. Bars represent P_{app} of pimozide under each condition (Fig 1A). The efflux ratio of pimozide was calculated from P_{app} under each condition (Fig 1B). Data are shown as mean ± SEM (n = 3). P : Pimozide 10 μM. S : Sertraline 500 μM. A : Aripiprazole 15 μM. V : Verapamil 100 μM . **, $p < 0.01$ compared with pimozide.

ATPase assay for P-gp substrate

We investigated the pimozide concentration-sensitive ATPase activity of P-gp (Fig 2). Concentration-dependent elevation of ATPase activity was initially observed, though concentrations of pimozide over about 10 μM decreased the ATPase activity.

Fig 2. ATPase activity of pimozide.

The results of ATPase activity assay are shown. Solid circles show activation study (n = 2) and open squares show inhibition study (n = 2). The black line represents the average and the gray line is the fitted curve. Data are given as relative activity (% of control). The pimozide concentration range is from 0.04 μM to 100 μM.

Efflux study of pimozide

The concentration dependence (in the range of 0.01 to 100 μM) of pimozide efflux from LLC-GA5-COL150 cells is shown in Fig 3. The J_{max}, K_t and k_d values estimated from the efflux assay data according to the equation (1) given in Materials and Methods were 84.9 ± 8.9 pmol/min/mg protein, 10.6 ± 4.7 μM and 0.67 ± 0.14 pmol/min/mg protein, respectively.

Fig 3. P-gp-mediated efflux transport of pimozide in LLC-GA5-COL150 cells.

The efflux rate of pimozide from LLC-GA5-COL150 cells are shown. The black line represents the efflux rate of pimozide from LLC-GA5-COL150 cells. The broken lines represent the saturable (・・・・) and non-saturable (-・-・-) transport components. The pimozide concentration range is from 0.01 μM to 100 μM.

Transport study across P-gp-expressing LLC-GA5-COL150 cell monolayers

Sertraline and aripiprazole significantly decreased the efflux ratio in LLC-GA5-COL150 cell monolayers to 11.0 and 21.9 % of the control, respectively, compared with pimozide alone (Fig 4).

Fig 4. Efflux ratio of pimozide in LLC-GA5-COL150 cell monolayers.

The values of the efflux ratio of pimozide in the absence or presence of sertraline, aripiprazole or verapamil in LLC-GA5-COL150 cell monolayers are shown. Data are shown as mean ± SEM (n = 5~6). Values were normalized to the control. P : Pimozide 10 μM. S : Sertraline 500 μM. A : Aripiprazole 15 μM. V : Verapamil 100 μM. *, $p < 0.05$; **, $p < 0.01$ compared with pimozide.

Discussion

253 To evaluate the possibility of drug-drug interaction of pimozide with sertraline and/or
254 aripiprazole in the gastrointestinal tract, we performed a transport study with Caco-2 cells, which have
255 been widely used for experimental studies of gastrointestinal permeability. The ER of pimozide was
256 decreased significantly and the permeability was increased when sertraline or aripiprazole was co-
257 administered with pimozide, compared with the ER of pimozide alone (Fig 1B). Moreover, the ATPase
258 activity of P-gp-expressing inverted membrane vesicles was elevated in a pimozide-concentration-
259 dependent manner (Fig 2). However, the ATPase activity was reduced in the high concentration range,
260 suggesting that pimozide is both a P-gp substrate and an inhibitor at higher concentrations. Futhermore,
261 the efflux of pimozide from P-gpoverexpressing LLC-GA5-COL150 cells had both saturable and non-
262 saturable linear components (Fig 3). For the saturable carrier-mediated transport of pimozide, we
263 obtained J_{max} and K_t values of 84.9 pmol/min/mg protein and 10.6 μM, respectively. Therefore, J_{max}/K_t,
264 which reflects affinity for the transport carrier(s), is 8.0 μL/min/mg protein. J_{max} and K_t of
265 Rhodamine123 (Rho123), a well-known P-gp substrate, are 988 pmol/min/mg protein and 173 μM,
266 respectively [24], giving a J_{max}/K_t value of 5.7 μL/min/mg protein. Therefore, the affinity of pimozide
267 for P-gp may be similar to that of Rho123, although it should be noted that the experimental method
268 and conditions were not the same in the two cases. Pimozide was transported in the B-to-A direction
269 of LLC-GA5-COL150 cell monolayers, and sertraline and aripiprazole decreased the ER of pimozide
270 by about 90% and 80%, respectively (Fig 4). In the gastrointestinal tract, the effective concentration

of pimozide is estimated to be at least 9 μM and its K_t for P-gp was 10.6 μM, suggesting that P-gp-mediated drug interactions could occur. Importantly, because the experimental concentrations of sertraline and aripiprazole were set based upon the clinically used doses, it is likely that P-gp-mediated drug interaction in the gastrointestinal tract could occur in clinical situations when these drugs are co-administered with pimozide.

In conclusion, pimozide is a substrate of P-gp, and its absorption is increased by sertraline and aripiprazole. Our results suggest that elevated pimozide blood levels observed when the drug is administered in combination with sertraline and/or aripiprazole can explained at least in part by interaction at P-gp. Further in vivo studies seem warranted.

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Conflict of interest

The authors declare that there is no conflict of interest.

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296 Writing – original draft: Hiroki Morishita

297 Writing – review & editing: Takuo Ogihara

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References

301 [1] P. Mackin, Cardiac side effects of psychiatric drugs., Hum. Psychopharmacol. 23
302 Suppl 1(2008) 3–14.
303 https://doi.org/10.1002/hup.915 PMID: 18098218.

304 [2] J. Kang, L. Wang, F. Cai, D. Rampe, High affinity blockade of the HERG cardiac
305 K(+)channel by the neuroleptic pimozide., Eur. J. Pharmacol. 392 (2000) 137–140.
306 https://doi.org/10.1016/s0014-2999(00)00123-0 PMID: 10762666.

307 [3] M. Gulisano, P. V Cali, A.E. Cavanna, C. Eddy, H. Rickards, R. Rizzo,

Cardiovascular safety of aripiprazole and pimozide in young patients with Tourette syndrome., Neurol. Sci. Off. J. Ital. Neurol. Soc. Ital. Soc. Clin. Neurophysiol. 32 (2011) 1213–1217. https://doi.org/10.1007/s10072-011-0678-1 PMID: 21732066.

[4] Product Information: ORAP® Tablet, 18th edition (2019), Astellas Pharma Inc. Tokyo, Japan. http://www.info.pmda.go.jp/go/pack/1179022C1034_1_11/. Last accessed Jan/12/2020.

[5] https://www.mhlw.go.jp/stf/shingi/2r9852000002ndoo-att/2r9852000002ndq5.pdf. Last accessed Jan/3/2020. Ministry of Health, Labour and Welfare, Japan.

[6] E. Spina, J. de Leon, Clinical applications of CYP genotyping in psychiatry. J. Neural Transm. 122 (2015) 5–28. https://doi.org/10.1007/s00702-014-1300-5 PMID: 25200585.

[7] E. Spina, F. Pisani, J. de Leon, Clinically significant pharmacokinetic drug interactions of antiepileptic drugs with new antidepressants and new antipsychotics. Pharmacol. Res. 106 (2016) 72–86. https://doi.org/10.1016/j.phrs.2016.02.014 PMID: 26896788.

[8] J. Alderman, Coadministration of sertraline with cisapride or pimozide: an open-

label,nonrandomized examination of pharmacokinetics and corrected qt intervals in healthy adult volunteers, Clin. Ther. 27 (2005) 1050–1063. https://doi.org/10.1016/j.clinthera.2005.07.013 PMID: 16154484.

[9] F. Muller, M.F. Fromm, Transporter-mediated drug-drug interactions. Pharmacogenomics. 12 (2011) 1017–1037. https://doi.org/10.2217/pgs.11.44 PMID: 21787191.

[10] M. Takano, R. Yumoto, T. Murakami, Expression and function of efflux drug transporters in the intestine. Pharmacol. Ther. 109 (2006) 137–161. https://doi.org/10.1016/j.pharmthera.2005.06.005 PMID: 16209890.

[11] L.Z. Benet, The drug transporter-metabolism alliance: uncovering and defining the interplay. Mol. Pharm. 6 (2009) 1631–1643. https://doi.org/10.1021/mp900253n PMID: 19874004.

[12] B.J. Gurley, A. Swain, D.K. Williams, G. Barone, S.K. Battu, Gauging the clinical significance of P-glycoprotein-mediated herb-drug interactions: comparative effects of St. John's wort, Echinacea, clarithromycin, and rifampin on digoxin pharmacokinetics. Mol. Nutr. Food Res. 52 (2008) 772–779. https://doi.org/10.1002/mnfr.200700081 PMID: 18214850.

[13] J. Rengelshausen, C. Goggelmann, J. Burhenne, K.-D. Riedel, J. Ludwig, J. Weiss,

G. Mikus, I. Walter-Sack, W.E. Haefeli, Contribution of increased oral bioavailability and reduced nonglomerular renal clearance of digoxin to the digoxin-clarithromycin interaction. Br. J. Clin. Pharmacol. 56 (2003) 32–38. https://doi.org/10.1046/j.1365-2125.2003.01824.x PMID: 12848773; PMCID: PMC1884337

[14] J. Weiss, S.-M.G. Dormann, M. Martin-Facklam, C.J. Kerpen, N. Ketabi-Kiyanvash, W.E. Haefeli, Inhibition of P-glycoprotein by newer antidepressants., J. Pharmacol. Exp. Ther. 305 (2003) 197–204. https://doi.org/10.1124/jpet.102.046532 PMID: 12649369.

[15] J.-S. Wang, H.-J. Zhu, B.B. Gibson, J.S. Markowitz, J.L. Donovan, C.L. DeVane, Sertraline and its metabolite desmethylsertraline, but not bupropion or its three major metabolites, have high affinity for P-glycoprotein., Biol. Pharm. Bull. 31 (2008) 231–234. https://doi.org/10.1248/bpb.31.231 PMID: 18239278; PMCID: PMC2666302.

[16] Y. Nagasaka, K. Oda, T. Iwatsubo, A. Kawamura, T. Usui, Effects of aripiprazole and its active metabolite dehydroaripiprazole on the activities of drug efflux transporters expressed both in the intestine and at the blood-brain barrier., Biopharm. Drug Dispos. 33 (2012) 304–315.

362 https://doi.org/10.1002/bdd.1801 PMID: 22847220.

363 [17] W.A. van Vloten, Pimozide: use in dermatology., Dermatol. Online J. 9 (2003) 3.

364 PMID: 12639456

365 [18] T. Tomono, T. Machida, H. Kamioka, Y. Shibasaki, K. Yano, T. Ogihara, Entinostat

366 reverses P-glycoprotein activation in snail-overexpressing adenocarcinoma HCC827

367 cells. PLoS One. 13 (2018) e0200015.

368 https://doi.org/10.1371/journal.pone.0200015 PMID: 29979729; PMCID:

369 PMC6034804

370 [19] T. Ogihara, M. Kamiya, M. Ozawa, T. Fujita, A. Yamamoto, S. Yamashita, S.

371 Ohnishi, Y. Isomura, What kinds of substrates show P-glycoprotein-dependent

372 intestinal absorption? Comparison of verapamil with vinblastine. Drug Metab.

373 Pharmacokinet. 21 (2006) 238–244.

374 https://doi.org/10.2133/dmpk.21.238 PMID: 16858128.

375 [20] K. Morimoto, M. Nakakariya, Y. Shirasaka, C. Kakinuma, T. Fujita, I. Tamai, T.

376 Ogihara, Oseltamivir (Tamiflu) efflux transport at the blood-brain barrier via P-

377 glycoprotein., Drug Metab. Dispos. 36 (2008) 6–9.

378 https://doi.org/10.1124/dmd.107.017699 PMID: 17940134.

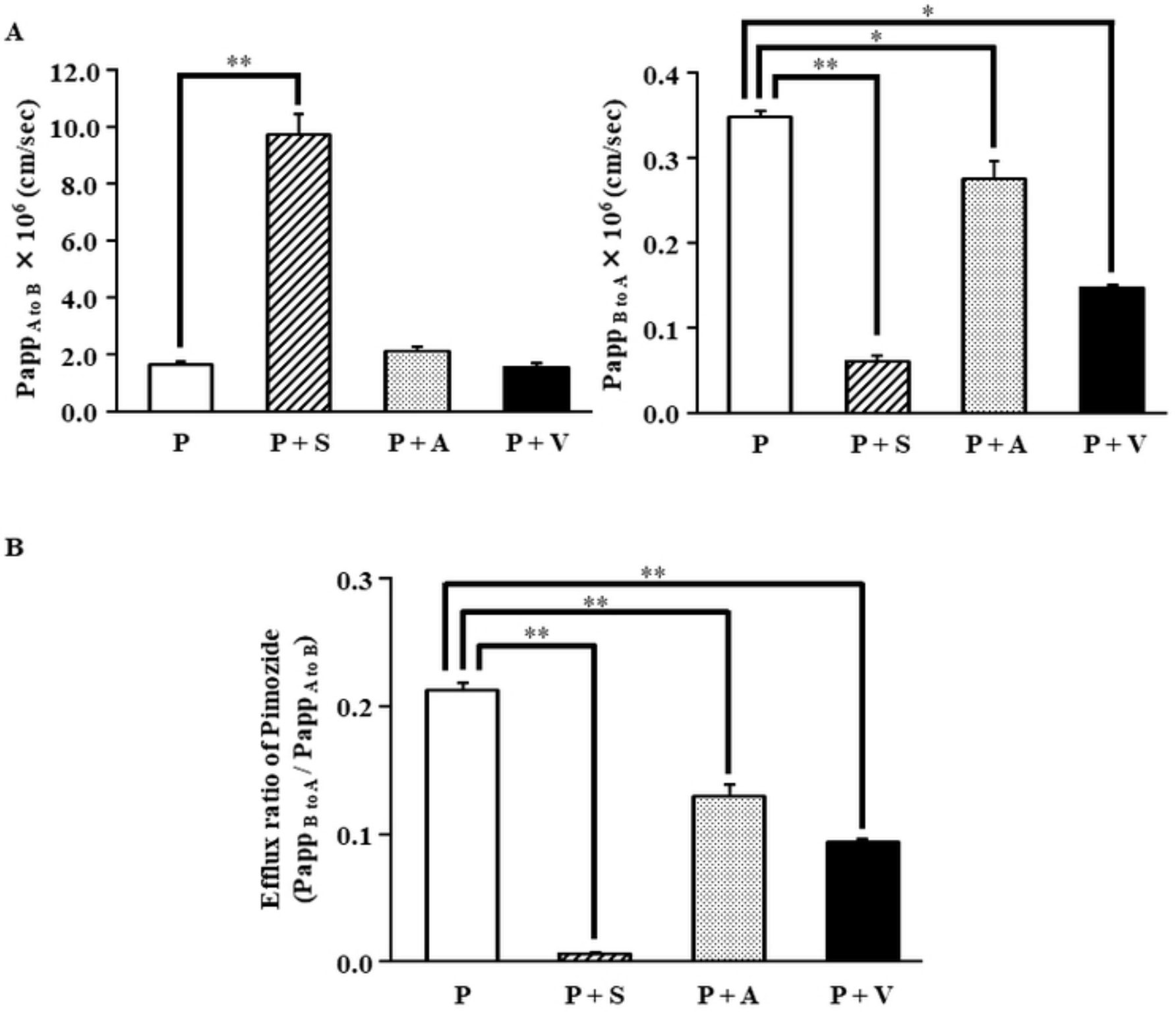
379 [21] K. Yano, S. Takimoto, T. Motegi, T. Tomono, M. Hagiwara, Y. Idota, K. Morimoto,

A. Takahara, T. Ogihara, Role of P-glycoprotein in regulating cilnidipine distribution to intact and ischemic brain., Drug Metab. Pharmacokinet. 29 (2014) 254–258. https://doi.org/10.2133/dmpk.dmpk-13-rg-072 PMID: 24366438.

[22] Food and Drug Administration. Guidance for Industry: Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System (2017). https://www.fda.gov/media/70963/download. Last accessed Jan/13/2020.

[23] T. Ogihara, I. Tamai, H. Takanaga, Y. Sai, A. Tsuji, Stereoselective and carrier-mediated transport of monocarboxylic acids across Caco-2 cells., Pharm. Res. 13 (1996) 1828–1832. https://doi.org/10.1023/a:1016081007981 PMID: 8987079.

[24] H.-J. Cho, M.-K. Choi, H. Lin, J.S. Kim, S.-J. Chung, C.-K. Shim, D.-D. Kim, Expression and functional activity of P-glycoprotein in passaged primary human nasal epithelial cell monolayers cultured by the air-liquid interface method for nasal drug transport study., J. Pharm. Pharmacol. 63 (2011) 385–391. https://doi.org/10.1111/j.2042-7158.2010.01221.x PMID: 21749386.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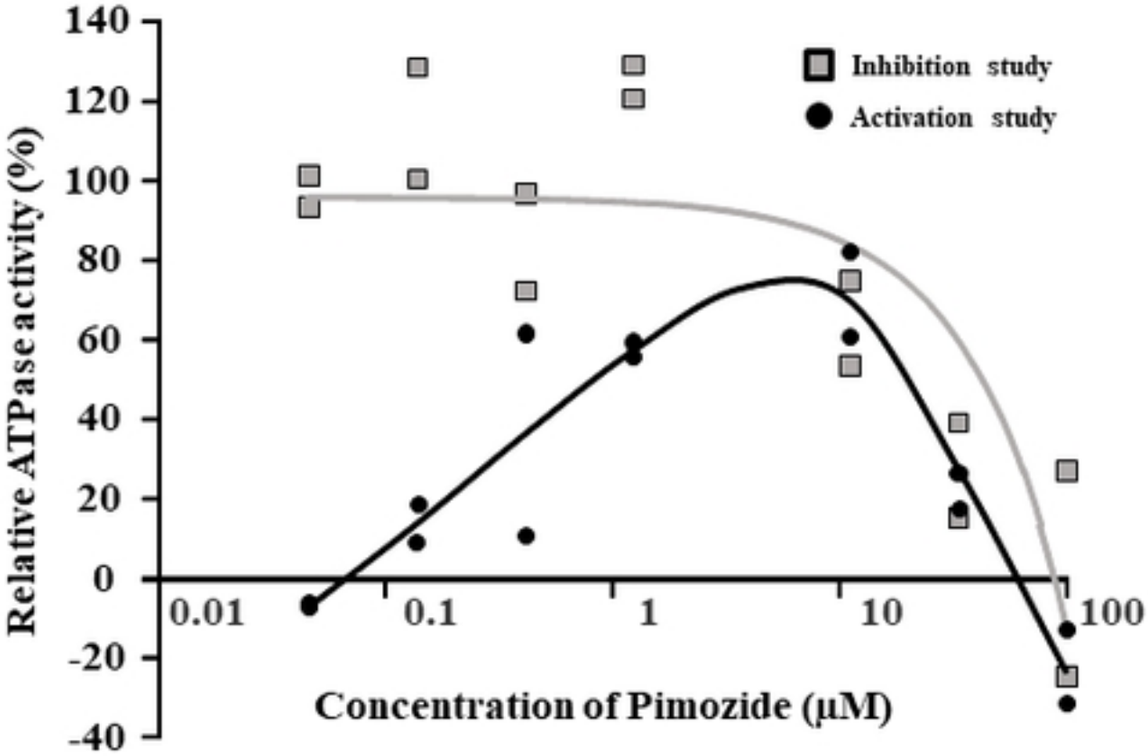
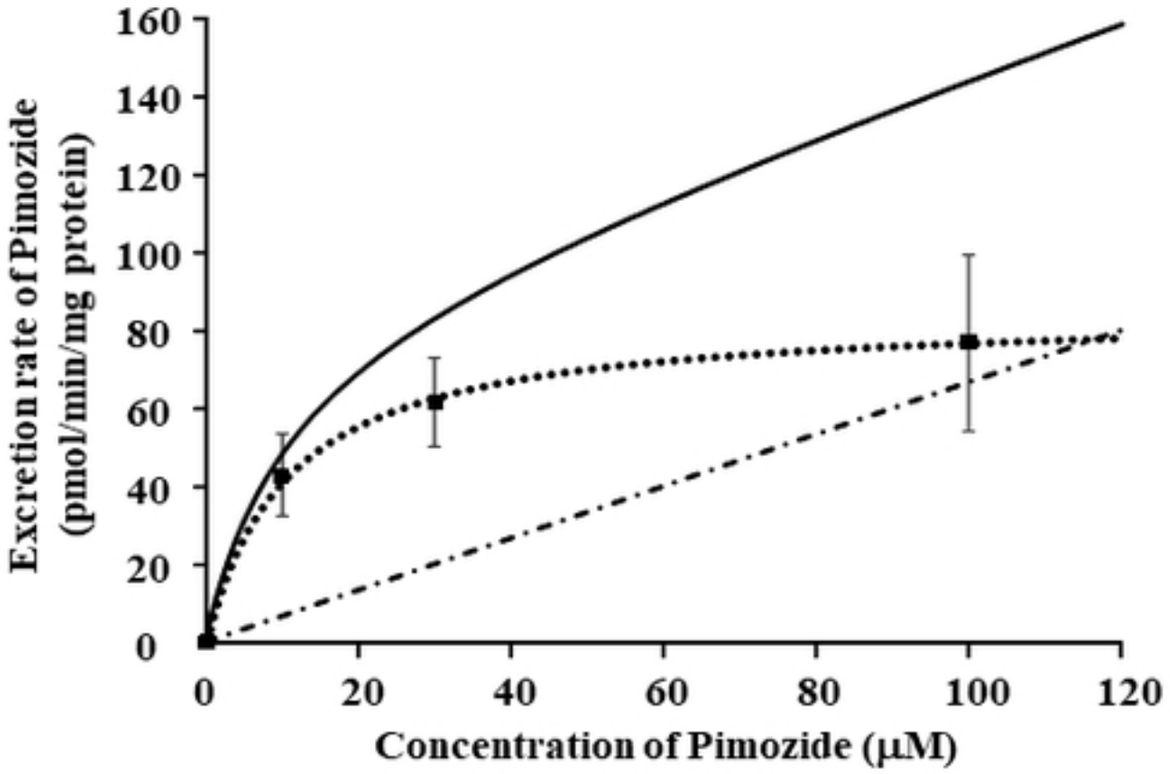
P+S
P+A
P+V
B
**
**
**
0.3
0.2
0.1
0.0
Efflux ratio of Pimozide
(Papp B to A / Papp A to B)
P
P+S
P+A
P+V

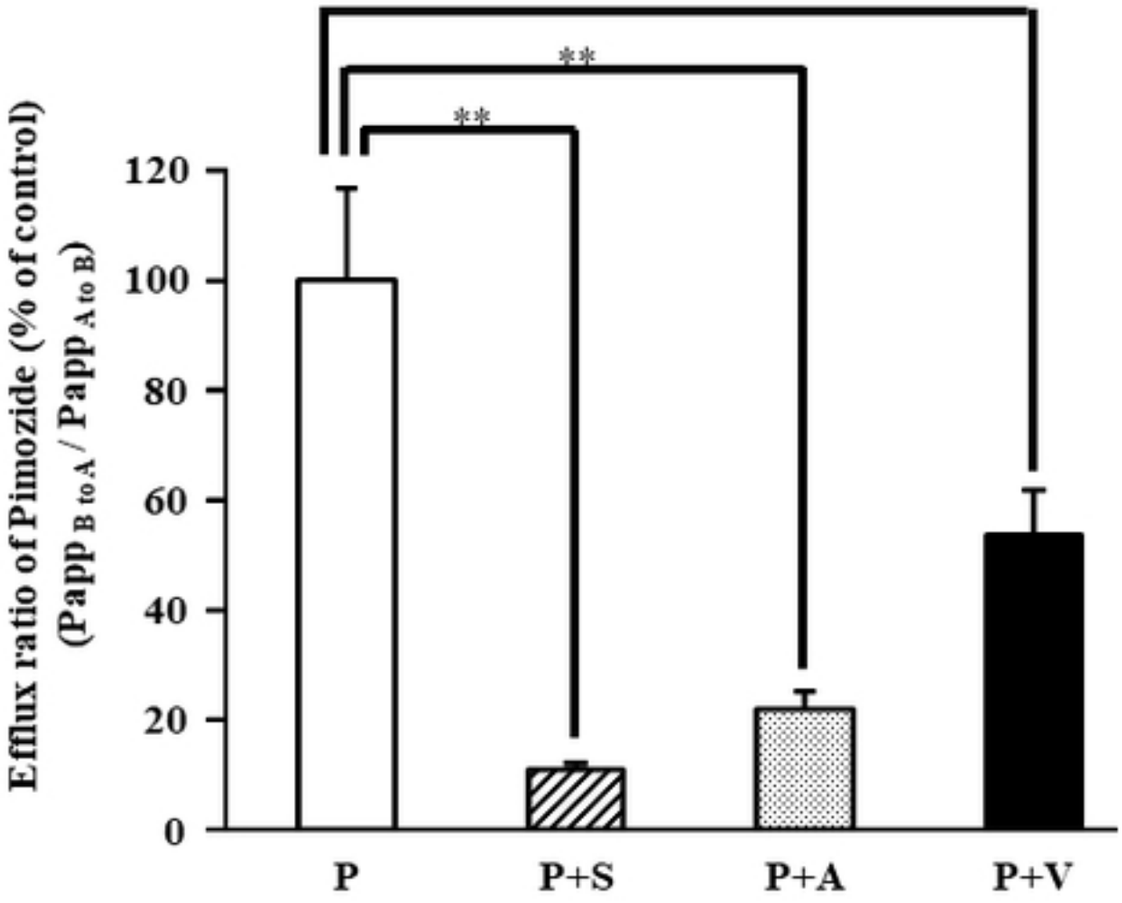
Figure


Inhibition study
Activation study
Relative ATPase activity (%)
140
120
100
80
60
40
20
0
-20
-40
0.01
0.1
1
10
100
Concentration of Pimozide (μM)

Figure


Excretion rate of Pimozide
(pmol/min/mg protein)
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Concentration of Pimozide (μM)

Figure


**
**
Efflux ratio of Pimozide (% of control)
(Papp B to A / Papp A to B)
120
100
80
60
40
20
0
P
P+S
P+A
P+V

Figure